Kinetics of *Pseudomonas fluorescens* inactivation with o*rtho*-phtalaldehyde

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Control of microbial growth is required in many microbiologically sensitive environments, particularly when wet surfaces provide favourable conditions for proliferation of microorganisms. An effective and wide spectrum disinfection strategy helps to overcome not only cross-resistance problems and existence of persister populations, but also the formation of recalcitrant and multi-resistant biofilms in disinfection dependent processes (Gilbert and McBain, 2003). According to the Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998, concerning the placing of biocidal products on the market; biocides are active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means. The biocide o*rtho*-phtalaldehyde (OPA) is an aromatic compound with two aldehyde groups that has been claimed to have an effective bactericidal character with potential for high-level disinfection (Simões et al. 2007). OPA has excellent microbiocidal, mycobactericidal and sporicidal activity, acting on multiple biochemical targets (Simões et al. 2006).

In this study, the antimicrobial effects of OPA at varying concentrations, pH and exposure times were assessed against *P. fluorescens* using respirometry. The data were modelled using the classical mechanistic model of Chick-Watson (CW) formulated as the integrated form: $\log_{10}(A_0/A) = kC^n t$, where *Ao* is the bacterial respiratory activity without OPA application; *A* is the respiratory activity of the after exposure to different OPA concentrations; *C* is the OPA concentration; *n* is the concentration exponent; *k* is the specific coefficient of inactivation; *t* is the exposure time. The CW log-linear model is mechanistic in the sense that it follows the principles of mass action.

The effect of OPA on the respiratory activity and consequent *P. fluorescens* viability was invariably more significant with increasing biocide concentration. For every OPA contact time and pH value, the biocide application caused the respiratory activity decrease. However, such a decrease was more pronounced when cells were suspended at pH 5 and 9. Concerning OPA contact time, its influence was only noticeable when comparing the results 5 and 60 min after biocide exposure ($p < 0.05$). The comparison of the data obtained 60 and 180 min after OPA exposure revealed no significant differences ($p > 0.05$). The bacterial inactivation data modelling by the CW method gave good log-linear regression coefficients (R^2 always higher than 0.739 – range values: 0.739-0.966). A susceptibility coefficient (*k*) of 87.9 ± 9.69 and a concentration exponent of 1.04 ± 0.0476 were associated with OPA inactivation effects. The results demonstrated that OPA was efficient in the inactivation of *P. fluorescens* cultures. The mechanistic CW disinfection model accurately described the OPA antimicrobial action for the tested conditions. This collection of experimental data on biocide antimicrobial action with the application of mathematical models can facilitate the development of more reliable antimicrobial strategies and the prediction of the outcome of biocide potential on microbial behavior.

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